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GENIC SIMILARITY OF AMERICAN AND EUROPEAN SPECIES OF THE LOBSTER *HOMARUS*

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In the course of investigating the population genetics of American lobsters (*Homarus americanus* M-E) and its implications for fisheries management and aquaculture, a survey of electrophoretically detectable protein variation was conducted (Tracey, Nelson, Hedgecock, Shleser and Pressick, 1975). This study revealed both a low level of genetic variation within populations relative to other invertebrates, and little genetic differentiation between populations.

In order to evaluate the potential of hybridization as a breeding program, it became important to examine genetic variation in the European lobster, *H. gammarus* (L), and to determine the degree of protein divergence between these two species of the genus. The results of this study are reported here.

The low level of protein divergence which was found, when contrasted with the morphological distinctness of the two species at all stages from egg to adult, has a bearing on the notion that protein evolution and biological (or organismal) evolution may proceed relatively independently of one another, at quite different rates (see, for example, King and Wilson, 1975).

Materials and Methods

Samples of *H. gammarus* from five localities were as follows (from North to South): 1) HGN—ten ovigerous females and ten males from Hordaland (Bergen), Norway, June, 1975; 2) HGS—four females and four males from Iona, Scotland, November, 1975; 3) HGI—six ovigerous and three nonovigerous females from various inshore localities, Ireland, October, 1975; 4) HGZ—eleven females and nine males collected 40 km south of Holyhead, Wales, in the Bay of Caernarvon, January, 1975; 5) HGW—one female and four males from the area of Aberdaron, Wales, November, 1975. Carapace lengths of these specimens ranged from about 85 to 118 mm.

Tissue preparation and electrophoretic techniques were the same as described for *H. americanus* (Tracey *et al.*, 1975). Muscle, heart, gonad, hepatopancreas, gill and green gland tissues were taken from all HGZ specimens and from seven HGN males and one HGN female. As the remaining individuals were needed for breeding purposes, only a single pereiopod was removed from each for electrophoretic analysis. Enzymes assayed in these leg tissue extracts (with numerical suffixes designating the loci scored on zymograms showing multiple zones of activity) were: esterase (-2, -8), fumarase (-1, -2), glyceraldehyde-3-phosphate dehydrogenase, hexokinase (-2, -3), malate dehydrogenase (-1, -2), 6-phosphogluconate dehydrogenase, phosphoglucose isomerase (-3, -4), phosphoglucomutase (-1), tetrazolium oxidase (-1, -2, -3, -5), and triosephosphate isomerase. In addition,

general proteins (-1, -2, -3, -4, -5), were revealed by the coomassie blue staining method.

After eye spots had developed in the egg masses of the ovigerous HGN and HGI females, egg samples were screened for protein variation as described previously for larval and juvenile stages (Hedgecock, Nelson, Shleser and Tracey, 1975). Due to the small amount of tissue in a lobster egg, it was necessary to collect two subsamples (eight to twelve eggs each) from each female in order to complete initial assays. The following enzymes were assayed in these progenies: esterase (-2, -4, -5, -6b, -8), fumarase (-1, -2), glutamate-oxaloacetic transaminase, glyceraldehyde-3-phosphate dehydrogenase (-1, -2), 6-phosphogluconate dehydrogenase, phosphoglucose isomerase (-3, -4), phosphoglucomutase (-1), tetrazolium oxidase (-2, -4, -5), and triosephosphate isomerase. Only the dark-staining protein -2 zone is routinely detected in egg samples. *Est-6b*, detected in egg, larval and juvenile whole animal extracts, is polymorphic in *H. americanus* (Hedgecock, Shleser and Nelson, 1976 and unpublished data). *H. gammarus* progenies surveyed here appear to be monomorphic for $Est\text{-}6b^{100}$, but due to the small sample size this locus is not included in calculations of genetic distance.

Tissue samples from three offshore American lobsters (GBS sample, Tracey *et al.*, 1975) were electrophoresed along with European samples in order to determine allozyme identities in the two species. Because of small sample sizes and lack of gene frequency differentiation, the four Irish Sea samples (HGS, HGI, HGZ, HGW) are pooled in the analysis.

Using Nei's statistics of genetic identity and genetic distance (Nei, 1972), pairwise comparisons between the Norway and Irish Sea samples and between these samples and eight *H. americanus* population samples studied previously are made for each locus and averaged.

Results

Seventeen of eighteen proteins assayed in *H. americanus* are demonstrable in *H. gammarus;* no activity is detected by the assay for leucine aminopeptidase. Zymograms for these proteins comprise a total of forty-one zones of staining activity that are either identical to or homologous with the zones described for *H. americanus*. Gene loci are symbolized by italicized abbreviations of enzyme names with numerical suffixes distinguishing among the multiple gene products detected on several zymograms (for a list of these see Tracey *et al.*, 1975). Alleles encoding the most common *H. americanus* allozymes in each zone are arbitrarily designated 100. *H. gammarus* allozymes and alleles are assigned numerals obtained by adding to or subtracting from 100 the number of millimeters separating a given allozyme from the common *H. americanus* allozyme after routine electrophoresis.

Genetic variability in H. gammarus *populations*

Full complements of nearly forty loci were studied only in eight Norway and twenty Irish Sea (HGZ) lobsters sacrificed for that purpose. Sample sizes for most loci were augmented by analyses of pereiopod tissues from additional adults reserved for broodstock and by estimation of paternal genotypes from progeny analyses. Thus, maximum numbers of independent genomes studied at some loci

in the Norway and Irish Sea samples are 60 and 96, respectively. Due to the variety of available sample material, however, average sample size per locus is considerably less than maximum—39 ± 3 genomes in the Norway sample and 57 ± 4 in the Irish Sea sample (Table I). Nevertheless, these samples are comparable to our previous *H. americanus* samples both with respect to numbers of genomes and, more importantly, for purposes of estimating genic heterozygosity and genetic distance (Nei and Roychoudhury, 1974), with respect to numbers of loci.

The amounts of genetic variability within the Norway and Irish Sea *H. gammarus* populations are essentially the same as those in *H. americanus*. Three measures of variability (average number of alleles per locus, proportions of polymorphic loci, and average proportions of heterozygotes per locus) agree closely with the *H. americanus* values (Table I).

In different *H. americanus* populations four loci, *Est-2*, *Pgi-4*, *Pgm-1* and *Tpi*, show a maximum of three alleles per locus; the remaining polymorphisms are diallelic. This is likewise true of variation in *H. gammarus*. *Mc* in the Irish Sea population sample is the sole triallelic polymorphism, and the average number of alleles per locus in *Homarus* populations is thus consistently 1.2.

Two measures of the proportions of polymorphic loci per population are also consistent in *Homarus* species. P, the proportion of loci showing any allozyme variation, is 20 ± 1%, and the more restrictive $P_{0.95}$ (see Table I footnote) is 14 ± 1%.

Finally, observed average heterozygosity (per locus or per individual) is 3.3% in Norway and 5.5% in Irish Sea populations. These rank lowest and ninth, respectively, among ten *Homarus* populations studied; thus, mean heterozygosity

TABLE I

Summary statistics of genetic variation in the European and American species of Homarus. *See text for detailed explanation of the Norway and Irish Sea population samples. Data for* H. americanus *are averages over eight population samples studied previously* (*Tracey* et al., *1975*).

	H. gammarus		*H. americanus*
	Norway	Irish Sea	
Number of loci studied	39	37	37.4 ± 1.8
Average number of genomes sampled per locus	39 ± 3	57 ± 4	59 ± 12
Average number of alleles per locus	1.20 ± 0.07	1.22 ± 0.08	1.23 ± 0.03
Proportions of polymorphic loci per population			
P*	0.205	0.189	0.209 ± 0.025
$P_{.95}$**	0.154	0.108	0.141 ± 0.015
Average proportions of heterozygotes per locus			
Observed (H_o)	0.033 ± 0.013	0.055 ± 0.025	0.039 ± 0.005
Expected (H_e)	0.040 ± 0.016	0.058 ± 0.026	0.046 ± 0.006

* P is the proportion of loci at which two or more alleles are detected.

** $P_{0.95}$ is the proportion of loci at which the most common allele has a frequency no greater than 0.95.

in the average lobster population is $4.0 \pm 0.4\%$ and expected heterozygosity averages $4.6 \pm 0.5\%$. The two European populations both show overall excess homozygosity as in *H. americanus* populations. Mean difference between observed and expected average heterozygosity, $d = -0.67\%$, is highly significant for the ten paired observations ($t = 5.49$, $P < 0.001$).

TABLE II

Allozyme variation at ten polymorphic loci in two populations of the European lobster, Homarus gammarus.

Gene	Population	Sample Size*	Allelic frequencies				Proportions of heterozygotes	
							Observed	Expected**
Acph-1			*98*	*100*				
	Norway	16	0.19	0.81			0.12	0.32
	Irish Sea	36	0.31	0.69			0.28	0.44
Acph-2			*96*	*100*				
	Norway	16	0.93	0.07			0.14	0.14
	Irish Sea	16	1.00	—			—	—
Acph-5			*99*	*100*				
	Norway	16	1.00	—			—	—
	Irish Sea	40	0.58	0.42			0.55	0.50
Est-2			*99*	*100*				
	Norway	32	0.72	0.28			0.31	0.42
	Irish Sea	36	0.53	0.47			0.50	0.51
Me			*98*	*100*	*102*	*104*		
	Norway	14	—	—	0.93	0.07	0.14	0.14
	Irish Sea	38	0.08	0.39	0.53	—	0.58	0.58
Pgi-3			*95*	*100*				
	Norway	60	0.02	0.98			0.03	0.03
	Irish Sea	40	—	1.00			—	—
Pgi-4			*96*	*98*				
	Norway	60	0.07	0.93			0.13	0.13
	Irish Sea	68	0.02	0.98			0.03	0.03
Pgm-1			*100*	*103*				
	Norway	60	—	1.00			—	—
	Irish Sea	46	0.02	0.98			0.04	0.04
Pgm-2			*95*	*100*				
	Norway	16	0.19	0.81			0.38	0.33
	Irish Sea	38	—	1.00			—	—
Tpi			*100*	*107*				
	Norway	60	0.98	0.02			0.03	0.03
	Irish Sea	72	0.97	0.03			0.06	0.06

* Estimated number of genomes sampled (see text).

** Expected genotype proportions calculated by Levene's formulae for small samples (Levene, 1949).

Genetic similarity of Norway and Irish Sea H. gammarus *populations*

The two population samples of *H. gammarus* appear to have different allelic frequencies at several polymorphic loci (Table II). The most substantial divergences are at *Acph-5* (Nei's genetic identity, $I = 0.80$), and at the *Me* locus ($I = 0.79$), the latter a parallel to *Me* differentiation among *H. americanus* populations (Tracey *et al.*, 1975). Sample sizes of the *H. gammarus* populations are too small to permit much confidence in the allelic frequency estimates of Table II, but, on the whole, gene frequencies appear quite similar. Norway and Irish Sea samples are monomorphic for the same allele at an additional twenty-six loci. As a result, the average genetic identity of conspecific *H. gammarus* populations is $\bar{I} = 0.987$. For all conspecific lobster population comparisons $\bar{I} = 0.994 \pm 0.001$ and $\bar{D} = 0.006 \pm 0.001$.

Genetic divergence of Homarus *species*

Identity and genetic distance statistics were computed for all loci studied in all pairwise combinations of European and American lobster populations. The results are summarized under four classes of genetic divergence: (A) gene-enzyme systems monomorphic, or nearly so, for the same electromorph in both species; (B) gene-enzyme systems monomorphic, or nearly so, for different electromorphs; (C) polymorphic systems sharing the same allozymes in both species; and (D) polymorphic systems showing species differences in electrophoretic profile (Table III).

The majority of loci (30/41) show no variability or divergence between species, nine polymorphic loci show a wide range of I values, and only two loci show fixed or nearly fixed differences between the species. New alleles found to be specific to *H. gammarus* are *Acph-5*^{99}, *Me*^{98}, and *Me*^{104}, *Pgi-4*^{96}, *Pgm-2*^{95}, and *Tpi*^{107}.

Since both fixed differences are acid phosphatases, one might question whether these mobility differences are due to two loci or to one locus that encodes a

TABLE III

Distribution of gene-enzyme systems according to four classes of similarity in comparisons between European and American lobster populations.

	Number of loci	Range of I*
A. Loci fixed for same allele *Acph-4, Est-4, Est-5, Est-6, Est-8, Fum-1, Fum-2, Got, G-3pdh, Hk-1, Hk-2, Hk-3, Idh, Mdh-1, Mdh-2, Per, 6-Pgdh, Pgi-1, Pt-1, Pt-2, Pt-3, Pt-4, Pt-5, Pt-6, To-1, To-2, To-3, To-4, To-5, Tr-4.*	30	>0.99
B. Loci fixed for different alleles *Acph-2, Acph-3*	2	0.0–0.08
C. Polymorphic loci sharing alleles *Acph-1, Est-2, Pgi-3, Pgm-1*	4	0.4–1.0
D. Polymorphic loci having different allelic profiles *Acph-5, Me, Pgi-4, Pgm-2, Tpi*	5	0.0–1.0

* Nei's (1972) statistic of genetic identity.

polypeptide subunit common to both enzymes. ACPH-2 is specific to testis and migrates about 4 mm less than an ACPH-3 band specific to green gland tissue. In *H. gammarus*, both of these enzymes migrate 4 or 5 mm less than the *H. americanus* bands, so a single locus change is a reasonable hypothesis. However, one Norway male appeared to be heterozygous, $Acph\text{-}2^{96/100}$, indicating that this ACPH is dimeric and that the *H. americanus* allozyme occurs in European lobster populations at low frequencies. Since this individual was at the same time homozygous at *Acph-3* for the typical *H. gammarus* allele, the two ACPH's must be encoded by at least two loci.

Nei's (1972) statistics of I and D averaged over all loci and interspecific population comparisons are: $\bar{I} = 0.896 \pm 0.007$ and $\bar{D} = 0.110 \pm 0.007$. Under certain assumptions, D estimates the average number of electrophoretically detectable amino acid substitutions per protein since divergence of the two species (Nei, 1972). Thus, eleven such codon substitutions are estimated to have occurred for every 100 proteins.

Progeny studies

A minimum of eight eggs from each ovigerous female was examined for variants of each of the thirteen proteins listed above. Phenotypic variation within progenies was detected only in the Norway sample and only for esterase-2, triosephosphate isomerase, and phosphoglucose isomerases-3 and -4. Only TPI and PGI-3 F_1 phenotypes conformed to single locus genetic models, as follows.

Progeny from one HGN female showed two TPI phenotypes: the common single-banded phenotype and a three-banded pattern consisting of the common band, a band migrating 7 mm farther, and an intermediate, heavier-staining band (*i.e.*, the classic dimer heterozygote zymogram). The female, herself a triple-banded presumptive *Tpi* heterozygote, hatched homozygous and heterozygous progeny in a ratio of 20 : 15, not significantly different than 1 : 1. TPI electrophoretic mobility in *H. gammarus* populations is apparently controlled by a single locus having two alleles, Tpi^{100} and Tpi^{107}.

Another HGN female proved to be heterozygous at $Pgi\text{-}3^{95/100}$. Her progeny consisted of $Pgi\text{-}3^{95/100}$ heterozygotes and $Pgi\text{-}3^{100/100}$ homozygotes in the ratio 69 : 57, not significantly different than 1 : 1. This is in accord with observations of PGI-3 inheritance in *H. americanus* (Hedgecock *et al.*, 1975). However, apparent isozyme formation between PGI-3 and PGI-4 in these *H. gammarus* heterozygous F_1 was the first evidence of interaction between these two enzymes. Progeny with the $Pgi\text{-}3^{95/100}$ genotypes uniformly exhibited, in addition to the three banded PGI-3 heterozygous pattern and a doubling of the PGI-3 satelite band, a doubling of the lower band in the PGI-4 zone. Such PGI zymograms are observed in fishes that have a duplication of the *Pgi* locus (Avise and Kitto, 1973). For the American lobster PGI patterns this explanation is unsatisfactory since PGI-4 appears to be a monomer (Hedgecock *et al.*, 1975). PGI isozyme structure in lobsters requires further study.

Discussion

As a whole, the genus *Homarus* is characterized by a rather low level of gene-enzyme variation relative to other invertebrates. For both the European and

American species, there are on the average 1.2 alleles per locus, one out of five loci polymorphic per population, and one out of every twenty-five loci heterozygous per individual genome. Average heterozygosity for invertebrates tends to be much higher; Selander and Kaufman (1973) find an average proportion of heterozygous loci per individual of 15.1% for 24 invertebrate species. In more recent investigations of marine invertebrates, however, average heterozygosity does range from 1.1% (*Asterias vulgaris;* Schopf and Murphy, 1973) to 22% (*Tridacna maxima;* Ayala, Hedgecock, Zumwalt and Valentine, 1973).

Certainly, *Homarus* is not atypical when compared with other decapod crustacea. A survey of eleven species representative of most of the major subdivisions of the order Decapoda on the west coast of North America yields an average heterozygosity of about 5.8% (Hedgecock *et al.,* 1976, and unpublished data). In contrast to most other invertebrates that have been studied, decapods tend to be large, mobile, omniverous animals occupying vast geographical ranges. Lower heterozygosity in such animals is thus compatible with the hypothesis that genetic variability is not maintained in species pursuing "fine-grained" adaptive strategies (Levins, 1968; Selander and Kaufman, 1973). Since all decapods studied have similar temperate distributions, the observations are likewise compatible with the notion that homozygosity evolves in environments with temporally (seasonally) fluctuating trophic resources (Valentine, 1976).

Genic heterozygosity in *Homarus* is concentrated at four or five loci in each species; but with the exceptions of ubiquitous *Est-2* and *Acph-1* polymorphisms, the two sets of variable loci differ. Such a distribution of variability over loci is typically observed (Selander, 1976).

Slight, but significant, heterozygote deficiency with respect to Hardy-Weinberg expected proportions is consistently observed. One way such departures may arise is by pooling together in a sample individuals from two or more differentiated demes (Wahlund effect). On a broad geographical scale at least, *Me* allele frequency divergence provides more direct evidence that populations of both species are indeed subdivided.

Divergence between the species of *Homarus* is rather small compared to differences between other congeneric animal species that have been studied. Even among morphologically similar or indistinguishable sibling species genetic distance is substantial [$\bar{D} = 0.581$, in the *Drosophila willistoni* group (Ayala, Tracey, Hedgecock and Richmond, 1974); and $\bar{D} = 0.672$ between the naturally hybridizing sea stars, *Asterias forbesi* and *A. vulgaris* (Schopf and Murphy, 1973)]. However, like *Homarus,* some morphologically distinct species of fishes, rodents and annual plants are characterized by small $\bar{D}$ values, in the range 0.02 to 0.15 (see review of Ayala, 1976).

Thus, species differences in *Homarus* support the notion that structural gene evolution (as measured by electrophoresis) may proceed independently and at a different rate from evolution at more complex phenotypic levels (King and Wilson, 1975; Wilson, Maxson and Sarich, 1974; Wilson, Sarich and Maxson, 1974). Moreover, since rates of protein evolution appear to be proportional to time (*ibid;* Ayala, 1976; Carson, 1976), great similarity in the electrophoretic profiles of European and American lobsters ($\bar{D} = 0.11$) may reflect fairly recent evolutionary divergence. Crude estimates of a divergence time may be calculated either from

theoretical equations (Nei, 1972) or from observed rates of protein evolution (for example, Yang, Soulé and Gorman, 1974; Carson, 1976); for *Homarus* these range from 82,000 years by the former method to 2 million years B.P. by the latter and suggest a Pleistocene separation. It is plausible that geographic isolation of American and European lobster populations did occur as the result of Pleistocene glaciation. (Figure 1, CLIMAP Project Members, 1976, illustrates, for example, the compression of temperate zone habitat along the Atlantic margins of Europe and North America at the height of the most recent glaciation, 18,000 B.P.) Since *Homarus* is today absent from the waters around Greenland and Iceland (A. Gardarsson, University of Iceland, personal communication), the two species of lobster have apparently been isolated at least throughout the late Pleistocene and Recent periods. "No choice" laboratory matings have produced viable F_1 hybrids (J. Carlberg and J. Van Olst, California State University, San Diego, personal communication; personal observation). Although more thorough characterization of pre- and postzygotic reproductive isolating mechanisms must be made, gene exchange between the two species appears possible at least under culture conditions. Interspecific hybridization is thus implicated as a potentially important means of introducing genetic variability into lobster broodstock.

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Summary

European lobsters (*Homarus gammarus*) from the Norway coast and from the Irish Sea are examined for electrophoretically detectable genetic variation in seventeen functionally different proteins. Forty-one loci encoding these proteins are homologous with loci studied in a previous survey of eight populations of *H. americanus*.

Progeny hatched from ovigerous Norway females show variation in three enzymes, but Mendelian inheritance is confirmed only for triosephosphate isomerase and for one of the phosphoglucose isomerases. Complex PGI phenotypes are described.

The average amounts of genetic variability in European and American lobster populations appear to be equivalent. More than one allele is detected at 20% of the loci, the average number of alleles detected per locus is 1.2 and the average proportion of loci heterozygous per individual is 4.0%. While much less genically variable than other invertebrates, *Homarus* is not atypical when compared with eleven decapod species that average 5.8% heterozygosity. This is consistent with hypotheses relating genetic variability to adaptive strategy.

At thirty loci *H. gammarus* is monomorphic for the common *H. americanus* allele. Two acid phosphatase systems are fixed or nearly fixed for alternative alleles in the two species while the remaining polymorphic loci show various degrees of interspecific divergence. Unique *H. gammarus* alleles are detected at five loci but only contribute significantly to species differences at the *Acph-5*, *Me*, and *Pgi-4* loci. *Acph-1*, *Est-2*, *Pgi-3*, and *Pgm-1* are polymorphic for the same alleles in both species, but again, with various differences in allelic frequencies. In sum, average genetic identity and average genetic distance are: $\bar{I} = 0.896 \pm 0.007$ and $\bar{D} = 0.110 \pm 0.007$, respectively. Compared to the values for conspecific population comparisons, $\bar{I} = 0.994 \pm 0.001$ and $\bar{D} = 0.006 \pm 0.001$, it is clear that a small but significant amount of genetic divergence separates the European and American lobster.

Based on the premise that protein differences between existing species reflect the amount of time since they shared a common ancestor, it can be speculated that the European and American lobsters were isolated during the Pleistocene. The apparent weakness of reproductive isolating barriers suggests that these populations have evolved allopatrically.

Finally, quantification of species' genetic differences, together with recent successes in interspecific laboratory matings, implicates species hybridization as a potentially important breeding practice in lobster aquaculture.

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